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NDRI-PR 85-01 FEBRUARY 1985

# SUMMARIES OF RESEARCH FISCAL YEAR 1984

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NAVAL DENTAL RESEARCH INSTITUTE

Navai Medicai Research and Development Command Bethesda, Maryland



CONTRACTOR MEDICAL CONTRACTOR CON

# NAVAL DENTAL RESEARCH INSTITUTE NAVAL TRAINING CENTER, BUILDING 1-H GREAT LAKES, ILLINOIS 60088-5259

SUMMARIES OF RESEARCH Fiscal Year 1984

These summaries cover research carried out from 01 October 1983 through 30 September 1984.

This document has been approved for public release; its distribution is unlimited.

Approved and released by:

G. E. CLARK

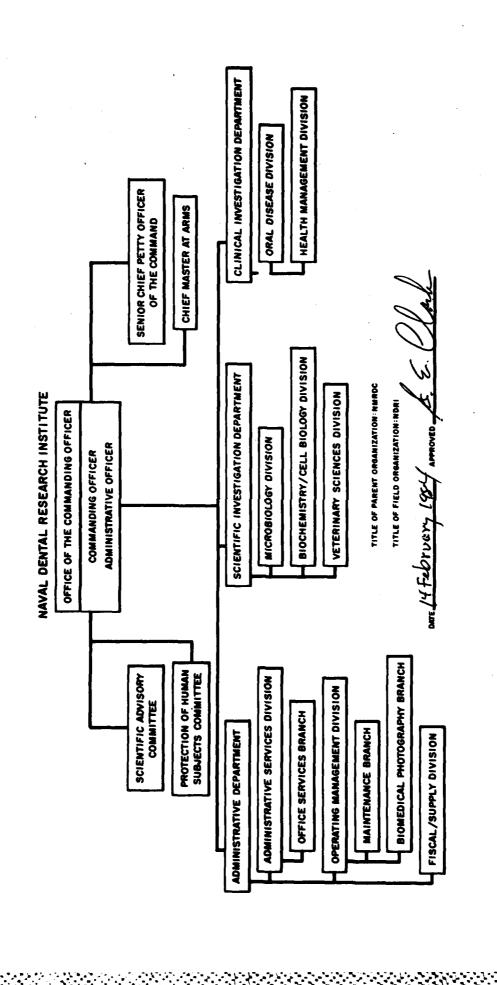
Captain, Dental Corps United States Navy

Commanding Officer

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#### COMMAND OVERVIEW

#### COMMAND

The Naval Dental Research Institute was officially established 01 January 1967 with an Officer-in-Charge. The Institute was developed from the Dental Research Facility, which was a Division of the Dental Department of the Naval Administrative Command, Naval Training Center, Great Lakes. The Institute became a fourth echelon command on 17 August 1969. The command is under the direction of the Naval Medical Research and Development Command.

MISSION

Naval Dental Research Inst,

The mission of the Institute is to conduct research, development, test and evaluation in dental and allied sciences, with particular emphasis on problems of dental and oral health in Navy and Marine Corps populations and on problems of fleet and field dentistry. This amual report includes summaries of significant accomplishments. The last year in both scientific and clinical research.

PERSONNEL

As of 30 September 1984, there were billets for 12 commissioned officers, 15 civilian employees, and 18 enlisted members, including one U.S. Army Animal Care Technician.

# **ORGANIZATION**

The Institute has undergone reorganization since 1967. The current organization of three major Departments is reflected on the preceding page. The Scientific Investigations Department consists of the Microbiology, Biochemistry/Cell Biology, and Veterinary Sciences/Pathology Divisions. Respectively, they carry out required microbiological, serological and bacteriological analyses; biochemical studies of etiological agents and of host factors involved in oral disease; assistance, advice and preparation of specimens for histological analysis; and research in the field of laboratory animal medicine and dentistry. The Clinical Investigations Department conducts research related to prevention and treatment of infections, problems of dento-alveolar trauma and injury, and the delivery of optimal dental care for the naval population. The Administrative Department provides the Institute with supply and fiscal services; library, general clerical services and manuscript preparations; photography and graphics; dental equipment repair; and equipment and facility maintenance, as well as special fabrications and instrumentation support.

## CLINICAL INVESTIGATIONS DEPARTMENT

Maintenance of deployed personnel who are affected by dental emergencies in a functional status can be enhanced by more comprehensive guidance and supportive equipment for the independent duty corpsman. A system, using computerized guidance for determining appropriate diagnosis and treatment of dental emergencies, along with a companion instrument kit was designed for this purpose. The computer program underwent preliminary field testing on 200 emergency cases at facilities aboard the USS Dixon (AS-37) and at NDC San Diego. This preliminary program was then redesigned and formally structured with documentation to permit future reprogramming for use in a broad range of microcomputers, as may exist in remote duty assignments. To prepare for the more definitive field testing planned in submarine operations, collaborative sessions were conducted at the Naval Submarine Medical Research Laboratory.

Evaluation of existent dental emergency field kits (ADAL 209 and FSC 6545-00-927-4840) was completed, deficiencies identified and revisions recommended. Noteworthy upgradings include an electric handpiece system, and a periodontal ligamentary syringe. Hand instrument and supply armamentarium was also adjusted, and the present kit will support such emergency endodontic, periodontal, surgical and prosthetic treatment as may be defined either by computer or handbook guidance.

In order to effectively address readiness in terms of oral health factors, comprehensive dental epidemiology information specific to the Navy young-adult population is necessary. Understanding of the incidence, distribution, severity, natural history and operational detriment of dental diseases affecting naval personnel in their unique occupational situation is essential to intelligent management of Navy health care resources. Directing dental care delivery and preventive methods on the basis of such information can ultimately reduce the risk and expense of dental emergencies during times of mobilization. To accomplish such data capture, the design, development and testing of a prototype dental examination data entry system was completed in collaboration with Navy Dental Clinics at Great Lakes, Norfolk, Orlando and San Diego. This system is composed of a 6502-based microprocessor, a membrane touch pad input device, a monitor and a printer with Epson graphics capabilities. The assembly will permit all information from an unlimited number of dental examinations to be entered, stored on floppy disks, retrieved and printed out onto a modified 603 Standard Form. The prototype system is now being used in the Naval Indoctrination Center at Great Lakes.

The first epidemiology study to use this automated dental data capture system compares DMF levels for recruits who have and

# CLINICAL INVESTIGATIONS DEPARTMENT (Continued)

have not received orthodontic treatment. Expansion of the system to one which will permit not only collection of initial exam data, but also collection of treatment entries, is now underway. Finally, a method to store dental and medical radiographs via laser disk technology is being investigated.

Beyond epidemiological studies and recapturability of lost dental records, automated central storage can provide a reference source to significantly improve the accuracy and speed of forensic identification. Preliminary studies were conducted to determine the technical feasibility of an intradental microlabel and computerized numeral identification method. Magnetic substrate materials suitable for laser imprinting were found to be detectable when placed beneath both composite and amalgam restorations, with and without TMS pin retention. The materials selected have the property of selective magnetization. They can be made magnetic when the need to locate the label arises and, being placed intradentally, are exceptionally resistant to destruction.

It is a matter of no small concern in contingency planning, that pain of carious origin continues to be one of the most common reasons why naval personnel seek emergency dental treatment. Marked caries reductions in the population at large in the past 15 years are not reflected in current caries-related dental emergencies in the Navy. A study was completed at NTC Great Lakes which revealed caries to be responsible for 47% of non-working-hour dental emergencies (unrelated to recent treatment). In 1969-70 similar data showed caries-related problems to generate 53% of Navy and Marine Corps dental emergencies in Vietnam. To determine the extent to which carious conditions predispose man-hour losses from sick call visits by active duty Navy and Marine personnel during working hours. NDRI conducted two studies in clinics serving different populations. At a Great Lakes clinic serving predominantly service school students, out of 1020 sick call patients in 24 working days, 23.3% (238) reported because of symptoms. Caries was responsible for 26.5% (63) of these cases. At the Naval Dental Clinic, Norfolk, Virginia, which serves a mixture of shore-based and fleet personnel, the same percentage (23.3%) of 1398 sick call reportees (326) reported because of symptoms in 11 working days. Caries was responsible for 21.5% (70) of these cases.

During times of mobilization, it is not likely that all needed dental treatment can be rendered prior to deployment into combat. Vietnam data showed that only 16.6% of carious teeth could be restored in basic training. Intelligent readiness planning, therefore, should include investigations into methods to arrest established carious lesions prior to the onset of debilitating symptoms in field conditions. The ongoing study to

# CLINICAL INVESTIGATIONS DEPARTMENT (Continued)

assess the efficacy of fluoride gels in the arrestment of caries and periodontal disease attempted to use survivability of disease-associated microorganisms in plaque samples as evidence of efficacy. When 0.4% SnF<sub>2</sub> gel was used in a daily brushing regimen for ten days, initial trends showed encouraging microbial reductions. Although the microbial count for total organisms remained reduced, some key microbial subgroups, selected for enumeration because of their association with caries and periodontal disease, returned to baseline levels. In the face of considerable literature supporting action of SnF<sub>2</sub> against caries and periodontal disease, it was felt that microbial reduction alone would not suffice as an early indicator of efficacy.

Interference with microbial metabolism is suggested as one reason why topical fluoride regimens reduce caries. resistant to bactericidal action of fluoride agents may survive at altered levels of cellular physiology which lower the level of metabolic byproducts capable of destroying the healthy dentition. Such a byproduct is acid. Plaque acid level may be a measurement more indicative of an overall reduction of pathogenesis of the total plaque flora. This laboratory, in collaboration with the University of Indiana and the ADA, has assembled a system which appears suitable for measuring plaque acidogenicity. Plaque pH is measured in terms of millivolt readings from a bimetallic wire electrode (Beetrode, W.P. Instruments), after the subject has rinsed with a 10% sucrose solution. Differences in acidogenic response of plaque microbial aggregates which have been exposed to use of fluoride gels will also be sought as indicators of efficacy of agents studied for arrestment of caries and periodontal disease.

During the first six months of enlistment, approximately 50% of operative dental needs are treated in the recruit population. Teeth with deep carious lesions receive priority attention. Four-year longitudinal data in a study to determine the most expeditious, conservative methods for treating deep carious lesions revealed that routine treatment of deep lesions, as practiced at the Great Lakes Recruit Training Center, is quite effective and reliable. The success of the following three conservative treatment procedures were compared: incomplete removal of decay with an indirect pulp cap; all caries removed and no pulp exposure; and complete removal of decay with a pulp exposure which resulted in a direct pulp cap. Success rates for these three conservative treatments ranged from 88 to 92%. No significant differences could be demonstrated among the methods.

A study to determine feasibility of extending deep caries treatment to a larger number of personnel by using the less time-consuming method of stainless steel crown placement is underway, comparing it with the pin-retained amalgam method.

#### CLINICAL INVESTIGATIONS DEPARTMENT (Continued)

Two-year data shows equal failure rates of 6% for each method. Of more importance, however, is a reluctance of practitioners to take time from productivity to develop skill in stainless steel crown placement technique. The procedure is regarded as temporary. Speed, therefore is not developed in the method to parallel that of pin amalgam placement and the service is perceived to require as much chair time as pin amalgam placement.

If fear prevents personnel from having dental problems resolved, no amount of refinement and development of preventive and treatment methods will impact on their dental emergency rates. In order to better define this situation in naval personnel, a study was completed in which dental treatment anxiety and need was measured in a sample of naval recruits at the Great Lakes Naval Training Center. Their treatment demands were then tracked at subsequent duty assignments for a one-year period. Findings indicated that treatment delivery was unaffected by patient level of dental anxiety. Although there is some small number of persons who avoid treatment because of anxiety, it would appear that conventional methods of patient handling are reasonably successful in preventing fear-based treatment avoidance among newly enlisted personnel.

Dental work provided to personnel prior to deployment must be expeditious, durable and not be mission-jeopardizing in the remote event of failure. Bridgework that is bonded directly to enamel is particularly desirable for the military, because failure of the restoration will not expose sensitive dentin and thus not require emergency treatment. This conservative bridgework is being delivered with increasing frequency in Navy Dental Clinics. degree of bonding between resin and metal, however, is not well controlled. In order to develop a method for quality control of laboratory etching of metallic frameworks, this laboratory designed a reflection photometry instrument to measure the extent of etching and thereby optimize bonding between resin and metal. The assembly consisted of a tungsten light source, fiber optic cables, micropositioning devices, an irradiance probe and a digital photometer. The instrument was able to distinguish among discs of Rexillium III etched under varying conditions. It can identify sets of discs etched under conditions producing metallic surface textures deemed optimum for bonding. Subsequent bond strength studies were conducted in collaboration with the University of Maryland. Initial results correlating reflection values and bond strengths appear to substantiate the clinical utility of this instrument.

Maxillofacial wounds constitute at least 15% of combat injuries. NDRI was tasked by Naval Sea Systems Command (NAVSEA) to manage the development of a two-tier ballistic and heat protective maxillofacial shield, in collaboration with a civilian

# CLINICAL INVESTIGATIONS DEPARTMENT (Continued)

contractor. This shield will be compatible with the MCU-2/p gas mask and the Navy battle and phonetalker helmets, concurrently being designed for the NAVSEA full body coverage Battle Dress Program. NDRI's design and initial construction of the shield (Phase I) is nearly complete. Prototype fabrication (Phase II) will be completed in FY85. Specification testing will subsequently be performed in preparation for fleet operational testing and evaluation.

# SCIENTIFIC INVESTIGATIONS DEPARTMENT

The Microbiology Division has investigated the organism, Streptococcus mutans, on the basis of its role as the major etiologic agent of dental caries. Its cariogenicity has been associated with its ability to produce lactic acid and water-insoluble glucans. The water-insoluble glucans may be important in the initiation of caries due to their ability to aggregate S. mutans on tooth surfaces and to act as a diffusion barrier to the lactic acid produced in dental plaque. This study was the first to relate both of these virulence factors from the same organism to caries activity in naval personnel and laboratory animals. The results obtained could help determine the role and importance of each factor in the caries process.

Dental plaque samples from 137 caries-active and caries-free naval recruits were tested for the presence of  $\underline{S}$ . mutans and the organism's ability to synthesize lactic acid and water-insoluble glucans.

The <u>S. mutans</u> strains were isolated from 78 caries-free and 59 caries-active male naval recruits. The caries-free group never had dental caries, while the caries-active group had a past or current history of this disease. The <u>S. mutans</u> strains isolated from the above groups were characterized for their abilities to synthesize lactic acid and water-insoluble glucan and these properties were related to the subject's current clinical conditions (see Table 1).

Table 1

	Caries-Free N=78	Caries-Active N=59
Lactic acid micromoles LA/ug DNA	3.6 <u>+</u> 1.03	3.9 <u>+</u> 0.84
Water-insoluble glucan ug glucan/ug DNA	13.54 <u>+</u> 10.3	22.05 <u>+</u> 24.72*

<sup>\*</sup>These values were significantly different, p < 0.01.

The amounts of lactic acid synthesized by  $\underline{S}$ .  $\underline{\text{mutans}}$  isolated from both the caries-free and caries-active recruits were not significantly different. However, the amount of  $\underline{S}$ .  $\underline{\text{mutans}}$  water-insoluble glucans from the caries-active group was significantly greater than the amount of  $\underline{S}$ .  $\underline{\text{mutans}}$  glucans from the caries-free group.

ORMAL PRESENTATIONS MADE AT MEETINGS OF SCIENTIFIC SOCIETIES/GROUPS Continued)

#### PRIL

DIEHL, M. C., presented "Dental Automation Applications" to he Naval Dental Clinic at Orlando, Florida and the Naval Dental linic at Norfolk, Virginia.

DIEHL, M. C., presented "NBC Defense" to the Casualty Care ourse conducted by the Naval Dental Clinic, Great Lakes, Illinois.

#### AY

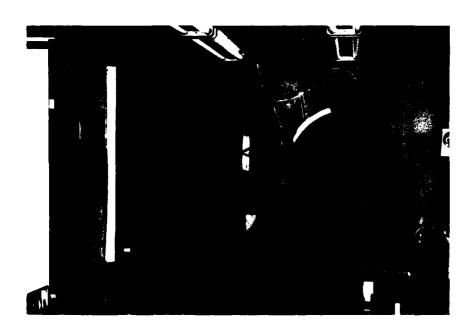
DIEHL, M. C., presented "Automated Oral Epidemiology nformation System" to the staff of the Naval Dental Clinic, an Diego, California.

CLARK, G. E., presented "Review of NDRI Research Program" to he staff of the Naval Dental Clinic, San Diego, California.

ESQUIRE, R. G., was a lecturer at the Preventive Dentistry nd Patient Motivation Course for Dental Officers at the Naval Lental Clinic, San Diego, California.

#### EPTEMBER

SIMONSON, L. G., presented "Glucanhydrolases and the Control of Glucans" at the Repligen Corp., Cambridge, Lassachusetts.



aptain D. T. Fenner touring the Marine Corps Expeditionary Dental Shelter with Captain G. E. Clark and CDR R. S. Baycar.

MAL PRESENTATIONS MADE AT MEETINGS OF SCIENTIFIC SOCIETIES/GROUPS entinued)

#### SRUARY (Continued)

McWALTER, G. M., presented "Overview of Surgical Endodontics" the Chicago Dental Society Midwinter Meeting, Chicago, Illinois.

#### RCH

BENNY, J. A., gave a presentation on Fitness Reports to the men Officers' Professional Network, Great Lakes, Illinois.

The following presentations were given at the 62nd General ssion of the International Association for Dental Research in llas, Texas:

- CLARK, G. E., "Pulp Testing as a Predictor of Conservative Deep Caries Treatment Success"
- COHEN, M. E., "Dental Treatment Anxiety and DMFS Status"
- DIEHL, M. C., "High Volume Computerized Transcription of Dental Examination Data"
- ESQUIRE, R. G., "Initial Response of Disease-Associated Dental Plaque Organisms to 0.4% SnF<sub>2</sub> Gel Brushing"
- KELLY, J. R., "Extent of Rexillium III Etching Determined by Reflection Photometry"
- LAMBERTS, B. L., "Effect of Fibronectin on Binding of S. mutans and S. sanguis to Hydroxyapatite"
- SHKLAIR, I. L., "Relationship of Lactic Acid and Glucans from S. mutans to Caries Activity in Naval Recruits"
- SIMONSON, L. G., "Effect of Serotype and Water-Insoluble Glucan Production on Accumulation of S. mutans"
- CLARK, G. E., presented "Current Concepts in Naval Dental search" at the Navy Breakfast held in conjunction with the sternational Association for Dental Research 62nd General ssion, Dallas, Texas.
- DIEHL, M. C., presented "High Volume Computerized anscription of Dental Examination Data" at the Chicago Section the American Association for Dental Research at Loyola niversity, Chicago, Illinois.

ESQUIRE, R. G., presented "Initial Response of Diseasesociated Dental Plaque Organisms to 0.4% SnF<sub>2</sub> Gel Brushing" at the Chicago Section of the American Association for Dental search at Loyola University, Chicago, Illinois.

# FORMAL PRESENTATIONS MADE AT MEETINGS OF SCIENTIFIC SOCIETIES/GROUPS

#### OCTOBER

DIEHL, M. C., presented "Design of an Automated Dental Epidemiology System" at the Second Annual Meeting of the American Association for Medical Systems and Informatics, Baltimore, Maryland.

DIEHL, M. C., presented "A Dental Trauma Diagnostic Program" and "Development of a Navy Dental Epidemiology Information System" at the Seventh Annual Symposium on Computer Applications in Medical Care, Baltimore, Maryland.

McWALTER, G. M., presented "Replantation of Avulsed Teeth" and "Intentional Replantation" at the University of Texas Dental School, San Antonio, Texas.

SIMONSON, L. G., led a meeting with the U.S. Food and Drug Administration Review Panel in Washington, D. C. regarding preclinical drug testing and IND requirements.

# FEBRUARY

DIEHL, M. C., presented a table clinic entitled "Computer Assisted Dental Examination" at the Chicago Dental Society Midwinter Meeting, Chicago, Illinois.



LCDR S. J. Patch discussing his research project with Captain R. P. Whitlock, DC USN.

#### OTHER PUBLICATIONS

- Aker, F., Schroeder, D. C., and Baycar, R. S. Cause and Prevention of Maxillofacial War Wounds: A Historical Review. Military Medicine 148(12):921-927, 1983.
- Baycar, R. S., Aker, F., Serowski, A., and Bailey, G. Mobile Oral Treatment and Examination Chair. Special Care in Dentistry pp. 224-225, 1983.
- Cohen, M. E., Cecil, J. C., Rocha, T. and McCarthy, T. P. Dental Recall Based on Caries risk. Military Medicine 148:928-931, 1983.
- Diehl, M. C. Design of an Automated Dental Epidemiology System. Proceedings of the American Association for Medical Systems and Informatics, 1983.
- Diehl, M. C. Development of a Navy Dental Epidemiology Information System. IEEE Proceedings of the Seventh Annual Symposium on Computer Applications in Medical Care, 1983.
- Hyman, J. J. and Diehl, M. C. A Dental Trauma Diagnostic Program. IEEE Proceedings of the Seventh Annual Symposium on Computer Applications in Medical Care, 1983.
- Kelly, J. R. and Cohen, M. E. Injectable Debris Associated with Dental Anesthetic Delivery. Journal of the American Dental Association 108:621-624, 1984.
- McWalter, G. M. Garre's Osteomyelitis of the Mandible Resolved by Endodontic Treatment. Journal of the American Dental Association 108:193-195, 1984.
- Wirthlin, M. R., Hancock, E. B. and Gaugler, R. W. The Healing of Atraumatic and Traumatic Incisions in the gingivae of monkeys. Journal of Periodontology 55:103-113, 1984.

# RESEARCH PROGRESS REPORTS - FY 1984

NDRI-PR 83-17	Glucanhydrolases and the Control of Glucans
NDRI-PR 83-18	Computerized Endodontic Diagnosis
NDRI-PR 83-19	Human Histologic Repair and Regeneration After Biologic Preparation of Diseased Root Surfaces
NDRI-PR 84-01	Summaries of Research - Fiscal Year 1983
NDRI-PR 84-02	Dental Recall Based on Caries Risk
NDRI-PR 84-03	Professional Papers Presented at the Seventh Annual Symposium on Computer Applications in Medical Care
NDRI-PR 84-04	Injectable Debris Associated with Dental Anesthetic Delivery
NDRI-PR 84-05	The Healing of Atraumatic and Traumatic Incisions in the Gingivae of Monkeys
NDRI-PR 84-06	Computerized Methods for the Graphic Representation of Multivariate Periodontal Data
NDRI-PR 84-07	Automated Dental Epidemiology System: III. Data System Design



Captain G. E. Clark and LCDR M. C. Diehl explaining the use of a computerized dental chart to Commodore R. G. Shaffer.

#### WORK UNITS - FISCAL YEAR 1984

- 61153N MR0412002-0445 The Pathogenesis of Oral and Dental Diseases
- 63706N M0095003-3028 Evaluation of New Methods to Arrest Oral Disease, and to Prevent and Treat Dental Emergencies in Naval Personnel

#### INDEPENDENT RESEARCH WORK UNITS

- 61152N MR000.01.01-0042 Evaluation of Dacron Reinforced Silastic as a Replacement for the Temporomandibular Joint Meniscus
- 61152N MR000.01.01-0048 Role of Trypsin Activity from Bacteroides gingivalis in Periodontal Disease of Naval Personnel
- 61152N MR000.01.01-0049 Prevalence of Dental Treatment Anxiety in Naval Personnel and Effects on Treatment Received
- 61152N MR000.01.01-0050 Role of Oral Microflora in the Chemical Degradation of Dental Luting Resins
- 61152N MR000.01.01-0051 Cellular Interactions and Stability of Fibronectin in the Oral Cavity
- 61152N MR000.01.01-0052 Coagglutination of Oral Disease Microorganisms from Naval Personnel



Captain E. B. Hancock, Colonel T. P. Sweeney and Dr. G. C. Battisone arriving at the Naval Dental Research Institute.

ADDENDUM TO SUMMARIES OF RESEARCH - Fiscal Year 1984

#### SCIENTIFIC INVESTIGATIONS DEPARTMENT (Continued)

influencing the attachment of bacterial cells to soft tissues. Recent work has shown that fibronectin favors the colonization of gram-positive bacteria to epithelial cells, but tends to inhibit such colonization by gram-negative organisms. Probing depths of periodontal sites have also been found to increase as gram-negative organisms, such as motile cocci and spirochetes, became the predominant microbial forms inhabiting these sites.

We have examined the stability of fibronectin in the presence of various oral microorganisms that are capable of producing proteolytic enzymes. Fibronectin stability was monitored by use of tritium-labelled fibronectin, prepared by reacting tritium-labelled formaldehyde with plasma fibronectin followed by borohydride reduction. The labelled fibronectin was incubated at 37 C for three hours with cell-culture suspension of 40 isolates. that included 25 gram-negative and 15 gram-positive oral The samples were electrophoresed on 4-30 percent organisms. gradient SDS acrylamide gels, which were then sectioned so that each sample lane was divided into two zones. Zone A, the upper third of a gel lane, contained intact fibronectin while the lower two-thirds of the lane, zone B, contained fibronectin fragments. Scintillation counts of the gel zones were used to assess fibronectin degradation. Degradative effects were strongest for the gram-negative organisms, especially isolates of Bacteroides gingivalis and Treponema denticola which yielded an 85 percent or more loss of label from zone A. Progressively weaker effects were observed for B. asaccharolyticus, B. intermedius, P. vulgaris, B. ochraceus, and P. aeruginosa, while fusobacteria did not degrade fibronectin to any detectable extent. By contrast, the gram-positive bacterial incubations did not result in any degradation except for moderate effects by S. faecalis and two L. casei isolates.

All of the strains were also tested for proteolytic activity on casein substrate plates, for cell aggregation with fibronectin, and for trypsin-like activity, using N- $\alpha$ -benzoyl-DL-arginine-p-nitroanilide (BAPNA) as substrate. All but one isolate showed proteolytic activity with casein, and no organism that could degrade fibronectin displayed cell aggregation when fibronectin was added to cell suspensions. Although a significant proportion of the strains degraded fibronectin under our test conditions, trypsin-like activity was observed only for the B. gingivalis isolates. These findings suggest that the degradation of fibronectin may be important for potentially pathogenic gram-negative organisms to colonize oral soft tissues. Such degradation may also have a bearing on periodontal pocket formation.

# SCIENTIFIC INVESTIGATIONS DEPARTMENT (Continued)

reduced adherence appreciably. Further exploration of carbohydrate/protein interactions was not pursued, since such interactions appeared to be relatively weak and less promising as means to exploit for the control of bacterial adherence than agents such as PGA and PAA.

We have also screened soil and compost organisms for their abilities to elaborate \( \alpha = 1 \), 3-glucanases that had a natural strong affinity for HA. Tests were conducted on about 40 bacterial isolates that had either been identified as glucanase-producers during earlier work or were more recently separated from soil or compost samples. Since none of the strains appeared to produce the enzyme constitutively, it was necessary in all cases to induce enzyme production. Although there was evidence for some of the isolates that at least part of their enzyme activity was cell-bound, extracellular enzyme activity was detectable with all However, the activities were extremely low. of the test trains. and were not increased appreciably when modification of culture conditions was attempted. The  $\alpha-1$ , 3-glucanases from several strains appeared to adhere weakly to HA at pH 5.5, but this binding was negligible in the neutral pH range. observations suggested the identification of the desired glucanases would require an extensive screening program, one beyond the scope of the present project.

Another study has focused on the possible influence of salivary components on bacterial adherence. This work was initiated on the basis of earlier investigations at NDRI that have shown that approximately 15-20 percent of caries-free recruits are free of S. mutans. Stimulated and unstimulated whole saliva samples were collected from 75 recruits, including 32 who were screened as caries-free upon initial examination and 43 who were designated caries-active. The caries-free subjects were also found to be free of, or to possess very low counts of, S. mutans, while the saliva samples from the caries-active group showed relatively high S. mutans counts. The samples were frozen immediately after collection. Samples were then selected from 20 of the caries-free subjects who were free of  $\underline{S}$ .  $\underline{mutans}$ , as well as from 20 caries-active subjects who showed at  $\underline{least}$  5,000  $\underline{S}$ . mutans counts per ml of saliva. These samples are presently being analyzed to determine whether they differ in their abilities to promote bacterial aggregation which could, in turn, affect adherence. The detection of salivary factors causing such effects could provide new information that might aid in the control of  $\underline{S}$ . mutans.

A different area of investigation has involved the stability of fibronectin in the oral cavity. Fibronectin is a glycoprotein that may function in the oral cavity by acting as a non-specific opsonin, by promoting adhesion of fibroblasts to collagen, or by

# SCIENTIFIC INVESTIGATIONS DEPARTMENT (Continued)

to these disks (p<.05), compared to control disks without the polypeptides. We have subsequently examined the effects of polypeptide application after the organisms had been permitted to attach to the HA disks, since this would more closely resemble the application of therapeutic agents in vivo. Tests conducted in this manner showed that PAA could displace all of the strains from saliva-coated hydroxyapatite (SHA) to a significant degree (p<.05). Additional experiments were then conducted in which S. mutans OMZ 176 was incubated in 50 mM sucrose in contact with SHA This strain was selected since it can synthesize far greater amounts of extracellular glucans from sucrose than the other test strains. It was found that PGA and PAA, as well as  $\alpha$ -1, 3-glucanase from NRRL 12324 <u>Pseudomonas</u> strain, now available at our laboratory, could all reduce the bacterial adherence significantly (p<.05). While the enzyme could degrade the glucans, the polypeptides evidently imposed their influence via electrostatic charge effects and competition for HA binding sites. The most pronounced result was obtained with a combination of PAA and the  $\alpha-1$ , 3-glucanase.

In another study, we investigated the effect of the S. mutans serotype source of water-insoluble glucans on adherence of S. mutans. Using a recently developed assay model, we measured the quantitative adherence of tritium-labelled S. mutans serotypes to uniform disks of water-insoluble glucan. When insoluble glucan disks from a serotype 'g' strain were used as the substratum,  $\underline{S}$ . mutans serotypes 'a' and 'd' showed the greatest amount of adherence, especially in the presence of sucrose. Disks were also prepared from water-insoluble glucans derived from S. mutans serotypes 'a', 'c', 'd', and 'g'. When these disks were exposed to radiolabelled serotype 'g' cells, no quantitative differences were detected. It appears that the relative adherence capabilities among the S. mutans serotypes is the result of differences in quantitative water-insoluble glucan-synthesizing capacities amoung the serotypes, rather than being due to any qualitative "specific recognition" of the various glucans by these oral streptococci. The presence of human saliva coatings on the glucan disks caused no significant differences in these experiments. In some cases the introduction of sucrose into the system caused agglutination of the suspended bacteria and there was a decrease in adherence. This is an artifact of the system which is caused by a decrease in the population of suspended particles available for adherence. However, many important answers to adherence questions were answered by this model.

The possible role of carbohydrate/protein interactions in the adherence of  $\underline{S}$ . mutans OMZ 176 to HA was investigated by tests with mannosidase and fucosidase. These enzymes degrade specific sugar components of salivary glycoproteins that might interact with cell-surface proteins. However, neither of the enzymes

# SCIENTIFIC INVESTIGATIONS DEPARTMENT (Continued)

1999 TEPROPRIES TRACTICAL CALLMANN BARROOM WORKERS WITH THE CALLMAN CALL

B. ochraceus, B. intermedius, B. macacae, or Streptococcus mutans, serotypes c and d (whole cell only).

Certain monoclonal antibodies from the second fusion were used in the development of a rapid coagglutination (CoA) procedure. Coagglutination (CoA) is a slide agglutination test for the rapid identification of bacterial antigens. In this procedure, Protein A, a cell surface fraction from Staphylococcus aureus that specifically binds to the Fc region of IgG, was bound to a specific IgG monoclonal antibody against Bacteroides gingivalis. When this reagent (Protein A and the monoclonal antibody) is mixed with an antigen, if it is specific for antigen, an agglutination reaction is visible (on a glass slide) within 1-3 minutes.

In a study carried out periodontal pocket samples were collected from 217 male naval recruits. Pocket depth ranged from 3-9 mm. The samples were grown on Wilkins-Chalgren agar, with a GN supplement, and incubated anaerobically for 5-7 days. Representative brown to black colonies were picked, purified, and characterized according to the procedures of Laughon, Syed and Loesche (J. Clin. Microbiol. 15:345, 1982). From these samples 11 (5.07%) gave positive results with CoA test; these strains were the only isolates that proved to be B. gingivalis according to biochemical tests. Some organims, i.e., Fusobacterium and Capnocytophaga strains, did give false positive reactions; these were the results of the organisms autoagglutinating. Strains of B. intermedius, B. melaninogenicus, B. asaccharolyticus, B. ochraceus and B. macacae, did not produce cross reactions using The use of monoclonal antibodies in the CoA test is the CoA test. a very specific, rapid and an easy-to-use method for distinguishing B. gingivalis from other Bacteroides strains. use of this procedure can also be easily adapted for the identification of other microorganisms.

The Biochemistry/Cell Biology Division has conducted several studies of bacterial initial attachment to and accumulation on hydroxyapatite (tooth enamel mineral), in an effort to identify means to interfere with these processes.

One such study examined the effects of the synthetic polypeptides, polyglutamic acid (PGA) and polyaspartic acid (PAA), on the adherence of tritium-labelled strains of Streptococcus mutans (NCTC 10449 and OMZ 176) and of Streptococcus sanguis (ATCC 10558 and 410) to hydroxyapatite. These polypeptides bind strongly to hydroxyapatite (HA) with a net negative electrostatic charge under neutral pH conditions. Earlier it had been reported that the pretreatment of HA disks with saliva containing PGA or PAA significantly decreased adherence of the test strains exposed

# SCIENTIFIC INVESTIGATIONS DEPARTMENT (Continued)

using pre-screened polyethylene glycol (PEG-4000). One protocol involved the use of Dulbecco's modified Eagle's medium (DMEM) while the other involved the use of Iscore's modified DMEM. The Iscore's medium was found to be superior for cell culture and supported a higher level of growth for both the myeloma and hybridoma cells. The culture plates were inspected for growth and antibody secretion ten days after fusion.

Two methods of cloning positive cultures were employed for both fusions. The limiting dilution technique was performed using spleenocytes from either BALB/c mice or from rice rats. We found that the rice rat spleenocytes provided a completely suitable feeder layer compared to the more expensive BALB/c spleenocytes. We now routinely use the rice rat spleenocytes for this technique. We found that limiting dilution did not provide "true" monoclones, even when the mathematical Poisson distribution of cells over the plates were provided. Therefore, we used soft-agarose cloning as an additional cloning technique. This technique is essentially foolproof if there is a good spread of clones on the agarose plate.

Once monoclonality was satisfied, we began to characterize the resultant antibodies. Using the enzyme immunoassay (EIA), the specificity of our monoclonal antibodies for both soluble and whole-cell antigens was studied. The first fusion resulted in antibodies that would only react with soluble antigen from B. gingivalis. It was monospecific in that no cross reactions were observed toward a panel of soluble antigens of closely related Bacteroides species. The monoclonal antibodies from this first fusion were not considered to be too useful, though, since they would not react with whole-cell surface antigens of B. gingivalis.

The second fusion was directed at both whole-cell and soluble B. gingivalis antigens. Using a slightly modified protocol monoclonal antibodies were produced that reacted with both the cell-surface and solubilized (particulate) antigens. Hybrids were visible after only six days following fusion. The optimal dilution appeared to be when 1 x 10 to 5 x 10 cells (based on original myeloma cell concentration) were cloned per well over a spleenocyte feeder layer. About  $14_{\mu}$  of 384 wells were positive for growth (67 of 96 positive at 5 x 10 well). It was found that monoclonal antibodies from this second fusion reacted with surface antigens by preparing fluorescent-antibody slides using an indirect staining technique. We labeled anti-mouse IgG and IgM with fluorescein isothiocyanate, using a conventional labeling procedure.

The monoclonal antibodies from the second fusion generally had a stronger reaction to the whole-cell antigen plates using the EIA assay procedure. There were no cross reactions with either

# SCIENTIFIC INVESTIGATIONS DEPARTMENT (Continued)

activity levels did not vary inordinately among isolates of the organism. The rim of one study was to investigate levels and variability of trypsin-like activity for various B. gingivalis strains during seven-day growth periods. Twelve strains of B. gingivalis, previously isolated from periodontitis patients, were grown anaerobically in a broth, and samples were removed for analysis after 24, 48, 72, 96, and 168 hours. Enzyme activity was determined using  $N-\alpha$ -benzoyl-DL-arginine-p-nitroanilide (BAPNA) as Bacterial counts were obtained from portions of the samples that were diluted, plated on agar, and incubated anaerobically for five to seven days. Mean enzyme activity increased by 2.5x from 24 hours to a maximum at 48-72 hours of  $4.1\pm1.4$  (mean  $\pm$  s.d.) BAPNA units/ $\mu$ l, after which it gradually declined to about two-thirds maximal value by 168 hours. maximal activities among the individual strains ranged from 2.3 to 6.3 BAPNA units/ $\mu$ l, and appeared to depend on the numbers of organisms present in the test samples. These observations indicate that activity measurements of the <u>B. gingivalis</u> trypsin-like enzyme may find application as useful determinants of periodontal disease status.

The presence of <u>B. gingivalis</u> in periodontal pockets has been a useful indicator of the severity of periodontitis. Currently it is cumbersome and time-consuming to isolate and identify the organism. A more rapid method for determining the presence of these organisms in a periodontal pocket would be with monoclonal antibodies produced against <u>B. gingivalis</u>. Direct tests using labeled monoclonal antibodies have been used to identify microorganisms in less than 15 minutes that previously required many days by culture methods. Experiments were carried out to produce monoclonal antibodies to <u>B. gingivalis</u>.

Two successful hybridoma fusions were performed. One series was aimed at producing monoclonal antibodies to water-soluble Bacteroides gingivalis antigens while the other fusion involved both whole cell and soluble antigens from this organism. Soluble antigens were prepared from cells grown in Wilkins-Chalgren broth supplemented with hemin and menadione. The cells were washed and then disrupted in an MSK cell homogenizer, and finally membrane-filtered. Whole cell antigens were prepared from cells grown as above but washed and fixed in formalinized saline.

Male and female eight-to-ten-week-old BALB/c mice were immunized with various combinations of soluble and whole cell antigens suspended in Freund's complete adjuvant (whole cell immunogens only). Two different fusion protocols were followed. Both involved growing a myeloma cell line 63-Ag8.653 (obtained from Dr. Kearney at the University of Alabama, Birmingham) to log phase immediately prior to fusion. Both protocols also involved

#### SCIENTIFIC INVESTIGATIONS DEPARTMENT (Continued)

It can be postulated that, if the <u>S. mutans</u> found in dental plaque synthesize high levels of water-insoluble glucans, the lactic acid produced will be trapped in the glucans and will, over a period of time, initiate a lesion. If, however, the <u>S. mutans</u> have a limited ability to synthesize water-insoluble glucans, the lactic acid will not be readily trapped but will be washed away or neutralized, resulting in limited caries activity. Lactic acid has long been accepted as one of the major virulence factors in caries initiation and development. However, the amount of lactic acid produced by <u>S. mutans</u> in this study, does not appear as correlated with dental caries as the amount of water-insoluble glucans synthesized. This nevertheless does not negate the importance of lactic acid in the decay process.

Twelve S. mutans strains, that synthesized various levels of lactic acid and water-insoluble glucans, were sent to Ohio State University for testing in germfree rats. Each strain was implanted into ten gnotobiotic animals, and the relationship of caries development to the synthesized amounts of lactic acid and water-insoluble glucans was determined. The organisms were reisolated at the termination of the animal phase of the study; the lactic acid water-insoluble glucans levels were again determined to see if the virulence factors changed while the organism was in the test animals.

Soluble and insoluble glucan levels ranged from 1.0 to 138 mg/mg DNA and from 3.0 to 160 mg/mg respectively. Lactic acid levels ranged from 1.1 to 4.0  $\mu$  moles/ $\mu g$  DNA. In the animals the buccal-lingual scores ranged from 1.2 to 17.2, and the total severity scores ranged from 67.8 to 107. Recovery of S. mutans from animals ranged from 110 x 10 to 198 x 10 colony forming units/mandibular quadrant.

The animal data was inconsistent and no relationship could be drawn between caries activity and the levels of lactic acid and water-insoluble glucan synthesized. Although the human clinical data showed a significant relationship of water-insoluble glucan to caries activity, this was not evident in the animal experiments. This may have been due to the animal model used. Rat teeth have deep fissures, and decay is often dependent on food impaction and the acid produced in the sulci. The role of the water-insoluble glucans, of aggregating the organisms and holding the acid at the plaque-tooth interphase is, in all probability, not as critical in rats as it is in humans.

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The organism <u>Bacteroides gingivalis</u> has been implicated in active gingival disease. The organism produces a trypsin-like enzyme that may enhance the organism's virulence. Measurements of activity of this enzyme in periodontal pocket samples could provide criteria of periodontal disease severity, so long as

# NDRI SEMINAR PRESENTATIONS FOR GREAT LAKES AREA NAVAL DENTAL OFFICERS

# **OCTOBER**

McWALTER, G. M., presented "Replantation of Avulsed Teeth" and "Intentional Replantation".

# DECEMBER

ESQUIRE, R. G., presented "Beginning Running" to the Naval Training Center Wellness Program.

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Dr. I. L. Shklair discussing microbiological techniques with RADM T. W. McKean.

#### OCTOBER

CLARK, G. E., attended the American Dental Association Annual Meeting in Anaheim, California.

CLARK, G. E., attended a Program Review meeting at the Naval Medical Research and Development Command, Bethesda, Maryland.

CLARK, G. E., attended the Illinois Section of the American College of Dentists dinner meeting, Chicago, Illinois.

ESQUIRE, R. G., attended a meeting to develop a pH measurement system at the University of Indiana, Indianapolis, Indiana.

ESQUIRE, R. G., attended the American Dental Association Conference on Food, Nutrition and Dental Health, Chicago, Illinois.

#### NOVEMBER

BENNY, J. A., attended the Women Officers' Professional Network meeting, Great Lakes, Illinois.

The Great Lakes Dental Society meeting was attended by the following personnel:

CLARK, G. E. DIEHL, M. C.



VADM L. H. Seaton, MC, USN touring the Microbiology Division with Captain G. E. Clark and Dr. I. L. Shklair.

# NOVEMBER (Continued)

A meeting of the Chicago Section of the American Association for Dental Research was attended by the following personnel:

CLARK, G. E. DIEHL, M. C. ESQUIRE, R. G.

LAMBERTS, B. L., attended biological chemistry seminars at the Chicago Medical School, North Chicago, Illinois.

#### **DECEMBER**

BAYCAR, R. S., attended the Combat Casualty Care Course (C-4) at Camp Bullis, San Antonio, Texas.

BENNY, J. A., attended the Women Officers' Professional Network meeting, Great Lakes, Illinois.

# **JANUARY**

BENNY, J. A., attended the Women Officers' Professional Network meeting, Great Lakes, Illinois.

The Chicago Section of the American Association for Dental Research meeting was attended by the following personnel:

BAYCAR, R. S. ESQUIRE, R. G. CLARK, G. E. KELLY, J. R.

The Great Lakes Dental Society meeting was attended by the following personnel:

CLARK, G. E. KELLY, J. R. DIEHL, M. C. SIMONSON, L. G. ESQUIRE, R. G.

DIEHL, M. C., attended a program coordination meeting at the Naval Health Research Center, San Diego, California.

LAMBERTS, B. L., attended a seminar on the Effect of Aging on Growth Hormone Secretion and Body Composition at the Chicago Medical School, North Chicago, Illinois.

SHKLAIR, I. L., attended the American Dental Association Foods, Nutrition and Dental Program of the Animal Resource Group, Chicago. Illinois.

# **FEBRUARY**

The Chicago Dental Society Midwinter meeting was attended by the following personnel:

BAYCAR, R. S. KELLY, J. R. CLARK, G. E. McWALTER, G. M. DIEHL, M. C. PATCH, S. J. ESQUIRE, R. G. SEROWSKI, A.

BENNY, J. A., attended the Women Officers' Professional Network Executive Board meeting, Great Lakes, Illinois.

BENNY, J. A., attended a Medical Service Corps meeting at the Chicago Medical School, North Chicago, Illinois.

The Great Lakes Dental Society meeting was attended by the following personnel:

CLARK, G. E. DIEHL, M. C. KELLY, J. R.



Colonel J. F. Taylor, VC, USA, touring the Animal Colony with Captain J. F. Cooper and PFC L. B. Thomas.

#### FEBRUARY (Continued)

The Illinois Society of Microbiology seminar at Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois was attended by the following personnel:

SHKLAIR, I. L. SIMONSON. L. G.

# MARCH

The 62nd General Session of the International Association for Dental Research in Dallas, Texas was attended by:

CLARK, G. E. McWALTER, G. M.
COHEN, M. E. LAMBERTS, B. L.
DIEHL, M. C. PEDERSON, E. D.
ESQUIRE, R. G. SHKLAIR, I. L.
KELLY, J. R. SIMONSON, L. G.

BAYCAR, R. S., attended the Alcohol/Substance Abuse Department course on the diagnosis, care and rehabilitation of patients with chronic alcoholism at the Naval Hospital, Great Lakes, Illinois.

BENNY, J. A., attended the Congress of the American College of Hospital Administrators in Chicago, Illinois.

BENNY, J. A., attended the Great Lakes luncheon for Medical Service Corps Officers.

CLARK, G. E., attended the Chicago Section of the American Association for Dental Research at Loyola University, Chicago, Illinois.

COHEN, M. E., attended a meeting of the Northeastern Illinois Section of the American Statistical Association, Chicago, Illinois.

The Great Lakes Dental Society meeting was attended by the following personnel:

DIEHL, M. C. ESQUIRE, R. G. KELLY, J. R.

SEROWSKI, A., attended the Design Engineering Conference sponsored by the American Society of Mechanical Engineers in Chicago, Illinois.

#### APRIL

BENNY, J. A., attended a meeting of the Hospital Administrator's Forum at the Chicago Medical School, North Chicago, Illinois.

#### APRIL (Continued)

- BENNY, J. A., attended a breakfast meeting of the Women Officers' Professional Network, Great Lakes, Illinois.
- BENNY, J. A., attended the Great Lakes Medical Service Corps Officers' luncheon, Great Lakes, Illinois.
- CLARK, G. E., attended the annual meeting and installation of officers of the Navy League, Great Lakes, Illinois.
- CLARK, G. E., attended a Commanding Officers' Conference at the Naval Training Center, Great Lakes, Illinois.

The Chicago Section of the American Association for Dental Research meeting at Northwestern University:

ESQUIRE, R. G. KELLY, J. R. SHKLAIR, I. L.

DIEHL, M. C., attended meetings on the Marine Corps Combat Casualty Information System at the Naval Health Research Center, San Diego, California.

HASTINGS, M. G., attended indoctrination meet ngs at the Naval Medical Research and Development Command, 1 thesda, Maryland.

LAMBERTS, B. L., attended the Lake Forest Chapter of Sigma XI meeting in Lake Forest, Illinois.

#### MAY

- CLARK, G. E., attended the Third Spring Joint National Congress, 1984, of the American Association for Medical Systems and Informatics. San Francisco. California.
- CLARK, G. E., toured the United States Army Institute of Dental Research, Letterman Army Institute of Research, San Francisco, California.
- CLARK, G. E., visited the Dental Service, Naval Hospital, San Diego, California.
- CLARK, G. E., visited and toured the Naval Health Research Center San Diego, California.
- SIMONSON, L. G., attended a lecture demonstration course on "Hybridoma/Monoclonal Antibody Production" at the Westin Hostel, Rosemont, Illinois.

# JUNE

BAYCAR, R. S., attended a conference on the face shield program review at Naval Sea Systems Command in Arlington, Virginia.

CLARK, G. E., attended a reception for VADM Seaton, Surgeon General United States Navy, at the CMO, Great Lakes, Illinois.

# JULY

CLARK, G. E., attended the Chicago Section of the American Association for Dental Research program planning meeting of the board, Chicago, Illinois.

The Academy of General Dentistry Annual Session in San Francisco, California, was attended by the following personnel:

ESQUIRE, R. G. KELLY. J. R.

HASTINGS, M. G., attended Introduction to Computers course in Chicago, Illinois.

KELLY, J. R., attended a workshop on Biocompatibility of Metals in Dentistry, American Dental Association, Chicago, Illinois.

#### **AUGUST**

SEROWSKI, A., attended the Society of the American Military Engineers, Great Lakes, Illinois.

#### SEPTEMBER

The Battle Dress Program Review Conference at Naval Sea Systems Command, Crystal City, Virginia, was attended by the following personnel:

BAYCAR, R. S. SEROWSKI. A.

The Great Lakes Dental Society meeting in Libertyville, Illinois was attended by the following personnel:

CLARK, G. E. ESQUIRE, R. G.

#### DISTINGUISHED VISITORS

#### OCTOBER

Captain R. F. McCullagh, MSC, USN, Administrative Officer, Naval Medical Research and Development Command, Bethesda, Maryland.

Dr. S. Murkejee, University of Illinois Dental School, Chicago, Illinois.

Dr. A. D. Steinberg, University of Illinois Dental School, Chicago, Illinois.

Captain R. J. Biersner, Naval Medical Research and Development Command, Bethesda, Maryland.

Dr. E. Postow, Naval Medical Research and Development Command, Bethesda, Maryland.

#### DECEMBER

Captain E. B. Hancock, DC, USN, Oral and Dental Health Program Manager, Naval Medical Research and Development Command, Bethesda, Maryland.



Commander Hargraves of the British Royal Navy touring the Computer Center with LCDR M. C. Diehl.

#### **JANUARY**

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HMCM Louis V. Green, Jr., Force Master Chief, Naval Medical Command, Washington, D. C.

Captain J. F. Kelly, DC, USN, Commanding Officer, Naval Medical Research and Development Command, Bethesda, Maryland.

Mr. Michael P. Jones, NSCSES, Norfolk, Virginia.

S. J. Schaberg, Dental Branch, Naval Medical Research Institute, Bethesda, Maryland.

#### FEBRUARY

Captain D. T. Fenner, DC, USN, Dental Officer, Headquarters, U.S. Marine Corps, Washington, D. C.

Captain R. P. Whitlock, DC, USN, Naval Medical Command, Washington, D. C.

Commodore R. M. Shaffer, Deputy Commander, Naval Medical Command, Washington, D. C.

Colonel J. F. Taylor, VC, USA, Naval Medical Research and Development Command, Bethesda, Maryland.

Captain E. B. Hancock, DC, USN, Oral and Dental Health Manager, Naval Medical Research and Development Command, Bethesda, Maryland.

Ms. Cecilia Dorsey, Evaluation Corporation, Arlington, Virginia.

Mr. Richard Platt, Evaluation Corporation, Arlington, Virginia.

Commander Hargraves of the British Royal Navy.

#### MARCH

Commander C. L. Lapp, CHC, USN, CREDO Center, Great Lakes, Illinois.

Dr. Jacob S. Hanker, Dental Research Center, University of North Carolina, Chapel Hill, North Carolina.

#### APRIL

Mr. Dennis Groat, President, Scherer Laboratories, Dallas, Texas.

#### APRIL (Continued)

Mr. J. B. Lewis, Vice President, Scherer Laboratories, Dallas, Texas.

CDR L. E. Heger, MSC, USN, Naval Medical Command, Washington, D. C.

Commodore L. E. Angelo, MSC, USN, Naval Medical Command, Washington, D. C.

#### MAY

The Naval Reserve Dental Company 213 toured the Institute while on active duty at the Naval Dental Clinic, Great Lakes, Illinois.

Ensign L. M. Candelaria, DC, USNR, Naval Dental Clinic, Naval Training Center, Great Lakes, Illinois.

Captain D. W. Turner, DC, USN (Ret.), Periodontics Department, Northwestern University Dental School, Chicago, Illinois.

Dr. C. Goodman, Periodontics Department, Northwestern University Dental School, Chicago, Illinois.

LT L. Williams, USA, Fort Sheridan, Illinois.

Mr. W. L. White, Miles Laboratory, Elkhart, Indiana.

Mr. H. T. Stephenson, Miles Laboratory, Elkhart, Indiana.

#### JUNE

VADM L. H. Seaton, Surgeon General, Washington, D. C.

RADM R. Milner, Naval Medical Command, Washington, D. C.

Ensign C. J. Cobb, DC, USNR, Naval Training Center, Great Lakes, Illinois.

Mr. S. Kirckheimer, Dentsply International, York, Pennsylvania.

Mr. E. Semen, University of Iowa, Iowa City, Iowa.

Mr. M. Jensen, University of Iowa, Iowa City, Iowa.

#### JULY

Mr. W. Ansite, Scott Aviation, Monrovia, California.

#### JULY (Continued)

- Mr. S. Koldhekar, Scott Aviation, Monrovia, California.
- Mr. L. Shields, Naval Sea Systems Command, Arlington, Virginia.
  - Mr. M. Jones, Naval Sea Systems Command, Arlington, Virginia.
- Ms. C. Dorsey, Evaluation Research Corporation, Arlington, Virginia.
- Captain J. Frazier, DC, USN, Dental Assignment Officer, Naval Military Personnel Command, Washington, D. C.
- Colonel C. W. DeLannoy, USAR, VC, Department of Animal Medicine, Michael Reese Hospital, Chicago, Illinois.
- Lieutenant Colonel E. W. Lindquist Jr., USA, Deputy for Veterinary Activities, MEDDAC, Fort Sheridan, Illinois.
  - Captain I. L. Rubin, USA, MEDDAC, Fort Sheridan, Illinois.
  - Dr. R. McLaughlin, AAALAC, Columbus, Ohio.
- J. L. Frazier, Naval Medical Research and Development Command, Bethesda, Maryland.
- Ensign Wardo, DC, USNR, Naval Dental Clinic, Naval Training Center, Great Lakes, Illinois.

Ensign Spradlin, DC, USNR, Naval Dental Clinic, Naval Training Center, Great Lakes, Illinois.

#### AUGUST

- Captain D. W. Turner, DC, USN (Ret.), Periodontics Department, Northwestern University Dental School, Chicago, Illinois.
- Colonel T. P. Sweeney, Commanding Officer, U.S. Army Institute of Dental Research, Washington, D. C.
- Dr. G. C. Battisone, Scientific Director, U.S. Army Institute of Dental Research, Washington, D. C.
- Captain E. B. Hancock, DC, USN, Oral and Dental Health Manager, Naval Medical Research and Development Command, Bethesda, Maryland.
- Colonel J. Taylor, VC, USA, Special Assistant for Veterinary Medicine, Naval Medical Research and Development Command, Bethesda, Maryland.

#### SEPTEMBER

RADM T. W. McKean, DC, USN, Inspector General, Naval Medical Command, Washington, D. C.

Dr. S. Hoff, Pharmacology Department, Chicago Medical School, North Chicago, Illinois.



Commodore Lewis E. Angelo, MSC, USN, visting with Captain G. E. Clark, Captain G. M. McWalter, and LCDR J. A. Benny.

#### OCTOBER

DN L. A. CASON reported for duty from the School of Dental Assisting and Technology, San Diego, California.

Captain G. E. CLARK was inducted as a Fellow of the American College of Dentists at the annual American Dental Association Meeting in Anaheim, California.

Dr. M. E. COHEN received a Quality Step Increase.

Dr. M. E. COHEN was promoted to Statistician (Health) GS-12.

Ms. C. McCANN joined the staff of the Office Services Branch of the Administrative Department.

Mr. E. D. PEDERSON received a Quality Step Increase.

Ms. M. J. ROUSE received a Quality Step Increase.

HM2 R. F. ROUSE was selected NDRI's Sailor of the Quarter.



Captain G. E. Clark presenting DTCS R. L. Douglass with a Letter of Commendation and a command plaque upon his retirement.

ONORS, AWARDS, POSITIONS HELD, CEREMONIES, STAFF ARRIVALS, EPARTURES, AND REENLISTMENTS (Continued)

#### **IOVEMBER**

LCDR J. A. BENNY was appointed Chairman of the By-Laws ommittee and as a member of the Executive Board of the Women fficers' Professional Network.

DTCS M. G. HASTINGS reported to NDRI for duty from the st Dental Battalion, 1st FSSG, FMFPAC, Camp Pendleton, alifornia.

#### **ECEMBER**

DN G. W. DALM was frocked to E-4.

DTCS R. L. DOUGLASS received a Letter of Commendation upon his etirement from active duty and transfer to the Fleet Reserve.

LT J. R. KELLY was appointed as AGD representative to NSI/ADA Subcommittee on base metal alloys.

Dr. B. L. LAMBERTS received a Merit Pay Performance Award.

Dr. I. L. SHKLAIR received a Merit Pay Performance Award.

Dr. L. G. SIMONSON was selected as Microbic ogy/Immunology ession co-chairman at the International Assoc ion for Dental esearch meeting.

#### ANUARY

DT3 T. J. BARNES was released from active duty.

DT1 S. M. BENSHOOF received the Navy League Award.

DT2 R. R. ROBERSON reported for duty from the National Naval lental Center, Bethesda, Maryland.

PV2 L. B. THOMAS, USA, was promoted to Private First Class.

DT2 P. K. TOMBASCO was selected NDRI's Sailor of the Quarter.

#### EBRUARY

 ${\tt DT1}$  S. M. BENSHOOF was nominated as NDRI's Sailor of the ear.

DN G. W. DALM was promoted to E-4.

#### MARCH

- DT3 J. A. JONES reported for duty from the Academy of Health Sciences, Fort Sam Houston, Texas.
- Dr. L. G. SIMONSON was elected IADR/AADR Microbiology/ Immunology Group Program Chairman.
- Ms. S. Y. WINN received a Letter of Appreciation for three years of continuous federal service.

#### APRIL

- LCDR J. A. BENNY received a Letter of Appreciation from the Commander Naval Medical Command Northeast Region for organizing and coordinating the effort to provide visual aids.
- PVT D. O. BROWN reported for duty from Walter Reed Army Medical Center, Washington, D. C.
  - PVT D. O. BROWN was promoted to E-2.
  - DT3 G. W. DALM was selected as NDRI's Sailor of the Quarter.

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HM1 B. E. JOHNSON received a Letter of Appreciation from the Commander Naval Medical Command Northeast Region for assistance in preparing a graphic presentation.

#### MAY

- DTCS M. G. HASTINGS received a Letter of Appreciation from the Commander Naval Training Center, Great Lakes, Illinois for assistance in coordinating plans for the Armed Forces Ball.
- Mrs. O. PATCH received a Letter of Appreciation from the Commanding Officer for volunteering as NDRI's Family Ombudsman from December 1982 to May 1984.
- PFC L. B. THOMAS, USA, received a Letter of Appreciation from the Commanding Officer upon his departure from NDRI.

#### JUNE

- DT1 S. W. BOCKOWSKI reported to NDRI for duty from NAMRU-3, Cairo, Egypt.
- LCDR M. C. DIEHL received a Letter of Commendation from the Commanding Officer upon his departure from NDRI for duty at 3D Dental Battalion, 3D FSSG FMFPAC, Okinawa, Japan.

#### JUNE (Continued)

- CDR R. G. ESQUIRE received a Letter of Appreciation from the Commanding Officer, Naval Dental Clinic, San Diego, California for outstanding contributions as a lecturer in the U.S. Navy's Preventive Dentistry and Patient Motivation Course.
- LCDR B. R. MERRELL received a Letter of Commendation from the Commanding Officer upon his departure from NDRI for duty at the Navy Environmental and Preventive Medicine Unit No. 6, Pearl Harbor, Hawaii.
  - LCDR S. J. PATCH resigned from the Navy.
- Dr. I. L. SHKLAIR was appointed Director, Scientific Investigations Department.
- Dr. L. G. SIMONSON graduated from Roosevelt University, Walter E. Heller College of Business Administration with a Master of Business Administration with honors.
- LT T. E. SOUTHARD reported for duty from the Branch Dental Clinic, Guantanamo Bay, Cuba.

#### JULY

- DT1 S. M. BENSHOOF reenlisted for four years.
- Major J. F. COOPER, BSC, USAF received a Letter of Commendation upon his departure from NDRI for duty at Wright Patterson Air Force Base.
  - DT1 S. R. HOEFS reenlisted for three years.
- Ms. C. McCANN resigned from the Office Services Branch, Administrative Department.
- $\,$  DT2 R. R. ROBERSON was selected as NDRI's Sailor of the Quarter.
  - HM2 R. F. ROUSE received a Good Conduct Medal.

#### **AUGUST**

Ms. H. E. SMITH joined the staff of the Office Services Branch, Administrative Department.

### HONORS, AWARDS, POSITIONS HELD, CEREMONIES, STAFF ARRIVALS, DEPARTURES, AND REENLISTMENTS (Continued)

#### SEPTEMBER

PV2 D. O. BROWN was promoted to E-3.

DT1 G. M. McKENDALL reported for duty from the U.S. Naval Mobile Construction Battalion Four, Rota, Spain.

CDR S. A. RALLS reported for duty from the Naval Medical Command Southwest Region, San Diego, California.



Captain G. E. Clark presenting Captain G. M. McWalter with a Letter of Commendation upon his retirement.

#### UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Date Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A154822	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) SUMMARIES OF RESEARCH - FISCAL YEAR 1984		5. Type of Report & Period Covered
		6. PERFORMING ORG. REPORT NUMBER  NDRI-PR 85-01
7. AUTHOR(a)		8. CONTRACT OR GRANT NUMBER(*)
9. PERFORMING ORGANIZATION NAME AND ADDRESS		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
Naval Dental Research Institute		ANEA WORK UNIT RUMBERS
Naval Training Center, Building 1-H		
Great Lakes, Illinois 60088-5259		
11. CONTROLLING OFFICE NAME AND ADDRESS		12. REPORT DATE
Naval Medical Research and Development		FEBRUARY 1985
Command, Naval Medical Command, National		13. NUMBER OF PAGES
Capital Region, Bethesda, MD 20814-5044		41
14. MONITORING AGENCY NAME & ADDRESS(If different from Controlling Office)		18. SECURITY CLASS, (of this report)
Commander, Naval Medical Command National Capital Region Washington, D. C. 20372		UNCLASSIFIED
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report)		

This document has been approved for public release; distribution unlimited.

#### 17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

This document has been approved for public release; distribution unlimited.

#### 18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Summaries of Research

#### 20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Brief summaries of research done from 1 October 1983 to 30 September 1984, including presentations, publications and distinguished visitors.

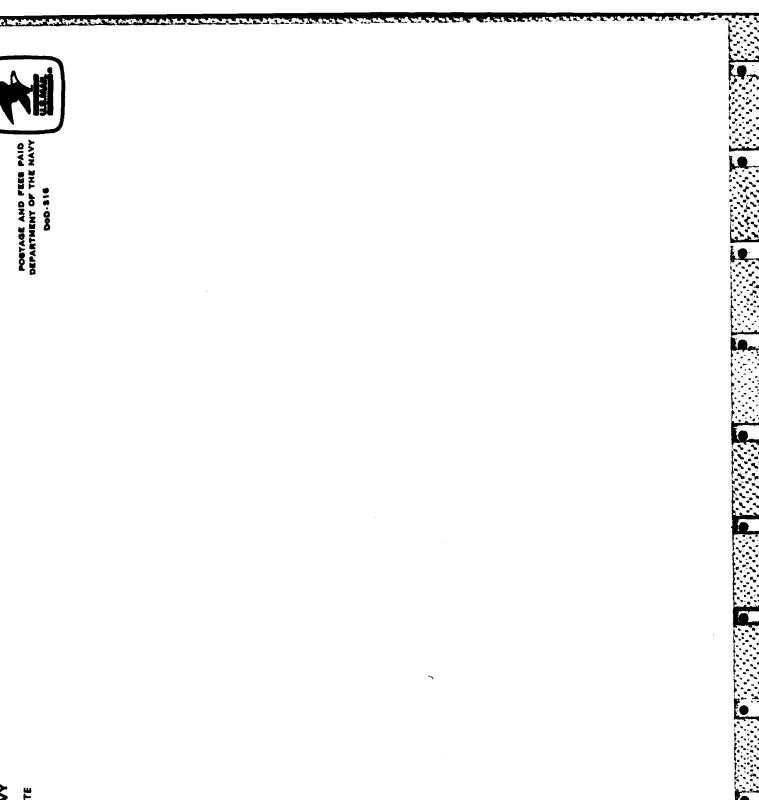
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